Serum Sex Hormone Levels Are Related to Breast Cancer Risk in Postmenopausal Women

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We conducted a nested case–control study to prospectively evaluate the relationship of serum estrogens and androgens to risk of breast cancer in postmenopausal women. From 1977 to 1987, 3375 postmenopausal women free of cancer and not taking replacement estrogens donated blood to the Breast Cancer Serum Bank in Columbia, Missouri. Of these, 72 were subsequently diagnosed with breast cancer. For each case, two controls matched on age and date and time of day of blood collection were selected using incidence density matching. The median age of subjects at blood collection was 62 years; the time from blood collection to diagnosis ranged from less than 1 to 9.5 years with a median of 2.9 years. Risk of breast cancer was positively and significantly associated with serum levels of estrogens and androgens. Compared to women in the lowest quartile, those in the highest quartile for non-sex hormone-binding globulin (non-SHBG) bound (bioavailable) estradiol had a relative risk of 5.2 (95% confidence interval [CI] = 1.5–18.5) and those in the highest quartile for testosterone had a relative risk of 6.2 (95% CI = 2.0–19.0). Our results lend considerable support to the hypothesis that serum concentrations of estrogens and androgens are related to the subsequent diagnosis of breast cancer in postmenopausal women. — *Environ Health Perspect* 105(Suppl 3):583–585 (1997)

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Introduction

Considerable data indicate that sex hormones play a major role in the etiology of breast cancer. Breast tumors frequently are hormonally dependent and a woman's reproductive history affects her risk (*1*). However, results of prospective epidemiologic studies that have evaluated the relationship of serum sex hormones to breast cancer have been inconsistent. In the largest prospective study of serum estrogens and postmenopausal breast cancer to date, Toniolo (*2*) reported significant positive associations of serum estradiol and estrone with risk. Berrino (*3*) also recently reported significantly higher serum testosterone levels among women who subsequently developed breast cancer. Nonsignificant excess risks of breast cancer among postmenopausal women with elevated serum estradiol, testosterone, and androstenedione also were reported by Helzlsouer (*4*). Others, however, have found no association between serum sex hormones and breast cancer risk (*5,6*). To further evaluate relationship of serum estrogens and androgens with breast cancer in postmenopausal women, we performed a prospective nested case–control study using the Columbia, Missouri Breast Cancer Serum Bank. This report summarizes our findings, which were published in their entirety in *Cancer Epidemiology, Biomarkers, and Prevention* (*7*).

Methods

The Breast Cancer Serum Bank, Columbia, Missouri, was established as part of the National Cancer Institute's Biological Markers Project to identify serum markers for breast cancer. A total of 7224 women who initially were free of breast cancer volunteered to donate blood and provide clinical information to the bank between 1977 and 1987. Follow-up by mail continued until 1989; but at least partly because of funding changes, 70% of the women were last contacted in 1982 to 1983. Informed consent was obtained from all women.

Women were eligible for the current study if they had at least 4 ml of blood remaining in the bank and if, at the time of blood collection, they had no history of cancer other than nonmelanoma skin cancer, were not diagnosed with benign breast disease within the previous 2 years, were postmenopausal, and did not report taking replacement estrogens. Of the 3375 women who met these criteria, 72 were diagnosed with histologically confirmed breast cancer. For each of these cases, two controls were selected from among the eligible women, using incidence density sampling. Controls were alive and free of cancer (except nonmelanoma skin cancer) at the age of the case's diagnosis and were matched to the case on exact age and on date (±1 year) and time (±2 hr) of blood draw. Following review of hormone results, one case and four controls were dropped because their follicle stimulating hormone (FSH) values indicated that they were premenopausal and one control was dropped because her hormone profile was consistent with exogenous estrogen use. That left 66 case–control sets with two controls and 5 case–control sets with one control for analysis.

Serum was stored at –70°C until analysis. Hormones were measured using commercially available radioimmunoassay (RIA) kits as follows: estradiol and testosterone (Diagnostic Products Co., Los Angeles, CA); estrone and androstenedione (Diagnostic Systems Labs, Webster, TX); dehydroepiandrosterone sulfate (DHEAS)

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Abbreviations used: CI, confidence interval; DHEAS, dehydroepiandrosterone sulfate; FSH, follicle stimulating hormone; RIA, radioimmunoassay; RR, relative risk; SHBG, sex hormone-binding globulin.

(ICN Biomedical, Costa Mesa, CA). Estrone sulfate also was measured by RIA after solvolysis, extraction of hydrolyzed estrone, and celite chromatography (*8*). Percent unbound and albumin-bound estradiol were measured using centrifugal ultrafiltration (*9*), and sex hormone-binding globulin (SHBG)-bound estradiol was calculated.

The relationship between serum hormones and breast cancer risk for the matched sets were evaluated using conditional logistic regression (*10*). Women were stratified into quartiles based on their hormone levels relative to the distribution of hormone values in controls, and a set of categorical (dummy) variables was included in models. To test for trends, models also were fit using quartile medians. Models also included time since menopause, height, weight, parity, and family history of breast cancer.

Results

Median ages of cases and controls at the time of blood collection were 61 and 62 years, respectively; and except for one control, all study subjects were white. Both cases and controls had a median age at menopause of 50 years. A slightly larger proportion of cases (20%) than controls (14%) were nulliparous. The median height of cases was 163 cm. This was significantly ($p = 0.02$) taller than controls, whose median height was 160 cm. Weights of cases and control did not differ, nor did their body mass indexes, which had a median of 26.0 for cases and 25.2 for controls. The proportions of cases and controls with a family history of breast cancer also did not differ. The median time from blood collection to diagnosis of breast cancer was 2.9 years with a range of less than 1 to 9.5 years.

As shown in Table 1, cases had higher mean serum levels of most hormones measured compared to controls, and differences were significant for androstenedione and testosterone. Analysis of relative risk (RR) also suggests relationship of serum estrogens and androgens with breast cancer. Women in the upper three quartiles of non-SHBG-bound estradiol had a 5-fold excess risk of breast cancer relative to women in the lowest quartile (Table 2). Similarly, women in the third and fourth (highest) quartiles of testosterone had RR values of 2.9 and 6.2, respectively. A significant trend of increasing breast cancer risk with increasing androstenedione level also was apparent. Although women in the highest quartile for DHEAS also were at a significantly elevated risk, there was no trend in risk of breast cancer with increasing level of this hormone.

Table 1. Geometric mean (± SD) levels of serum hormones in cases and controls.

	Cases, $n=71$	Controls, $n=133$	p Value[a]
Estradiol, pmol/liter	56.7 ± 4.4	48.6 ± 4.0	0.17
Non-SHBG bound estradiol, pmol/liter	25.8 ± 2.3	21.3 ± 1.9	0.13
Estrone, pmol/liter	127.5 ± 6.6	119.8 ± 5.2	0.38
Estrone sulfate, pmol/liter	510.4 ± 32.6	547.7 ± 28.3	0.41
Testosterone, nmol/liter	0.75 ± 0.07	0.55 ± 0.04	0.01
Androstenedione, nmol/liter[b]	3.52 ± 1.6	2.97 ± 1.5	0.02
DHEAS, μmol/liter	2.41 ± 0.21	2.01 ± 0.11	0.08

[a] p Value (2-sided) from Student's t-test. [b] One matched set deleted because case was an influential outlier.

Table 2. Numbers of subjects and adjusted relative risks[a] (95% confidence intervals) of breast cancer by quartile of serum estrogens and androgens.

	Quartile				
Hormone	1 (low)	2	3	4 (high)	Trend-p
Estradiol					
(No. cases, no. controls)	(6,32)	(24,31)	(26,36)	(15,34)	
Adjusted RR (95%CI)	1.0	4.9 (1.6–14.9)	4.7 (1.5–14.3)	2.7 (0.8–9.1)	0.12
Non-SHBG-bound estradiol					
(No. cases, no. controls)	(5,33)	(24,33)	(23,33)	(19,34)	
Adjusted RR (95%CI)	1.0	5.9 (1.8–19.3)	4.8 (1.5–15.7)	5.2 (1.5–18.5)	0.12
Estrone					
(No. cases, no. controls)	(14,31)	(17,34)	(16,34)	(24,34)	
Adjusted RR (95%CI)	1.0	1.2 (0.5–3.3)	1.0 (0.4–2.9)	1.8 (0.6–5.1)	0.36
Estrone sulfate					
(No. cases, no. controls)	(19,33)	(19,33)	(17,33)	(16,34)	
Adjusted RR (95%CI)	1.0	0.8 (0.3–2.1)	0.8 (0.2 -2.2)	0.8 (0.3–2.2)	0.58
Testosterone					
(No. cases, no. controls)	(9,32)	(13,28)	(20,39)	(29,34)	
Adjusted RR (95%CI)	1.0	2.9 (0.9–9.4)	2.9 (1.0–8.6)	6.2 (2.0–19.0)	0.002
Androstenedione					
(No. cases, no. controls)	(10,30)	(20,34)	(20,35)	(21,34)	
Adjusted RR (95%CI)	1.0	1.7 (0.6–4.7)	2.0 (0.8–5.3)	2.2 (0.8–5.8)	0.02[b]
DHEAS					
(No. cases, no. controls)	(13,33)	(19,31)	(8,34)	(31,35)	
Adjusted RR (95%CI)	1.0	1.6 (0.6–4.1)	0.6 (0.2–1.9)	2.8 (1.1–7.4)	0.05

[a] Cases and controls were matched on age, time, and year of blood collection and models included years since menopause, height, weight, family history of breast cancer, and parity. [b] One matched set deleted for trend test because case was an influential outlier.

To evaluate whether higher serum hormone levels in cases might be an effect rather than a cause of the cancer, we stratified cases into those diagnosed within 2 years of blood collection ($n = 25$) and those diagnosed more than 2 years after collection ($n = 46$). Except for testosterone, relationships between serum hormones and breast cancer did not differ markedly between the two groups. For testosterone, unadjusted RR among the women diagnosed close in time to blood collection were 2.5 (95% CI = 0.6–11.2) and 4.1 (95% CI = 0.9–18.0), respectively, for the middle and upper tertiles. Comparable RR values for the women diagnosed further in time from collection were 1.0 (95% CI = 0.4–2.7) and 1.3 (95% CI = 0.5–3.4).

Discussion

Results of this prospective study provide strong support for a relationship between serum sex hormone levels and the subsequent development of breast cancer in postmenopausal women. Women in the upper three quartiles of estradiol were at an elevated risk of breast cancer. The relationship was more consistent for non-SHBG-bound estradiol, the fraction that is biologically available, than for total estradiol. Serum levels of the androgens, testosterone, androstenedione, and DHEAS, all were positively associated with breast cancer risk. The association was strongest for testosterone, but we could not discern whether that association was a cause or an effect of the malignancy. Additional studies are needed to determine how serum hormone levels modify breast cancer risk in postmenopausal women.

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